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RESEARCH ARTICLE

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF VORINOSTAT USING RP-HPLC METHOD

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ABSTRACT

A simple and effective RP-HPLC method had been developed for the estimation of vorinostat in capsule**,** using Apollo C18 (4.6 x 150mm, 5µm), mobile phase 100% methanol, detection wavelength at 247 nm, at flow rate of 1ml/min at retention time 3.3 min for vorinostat. Linearity was obtained in the range of 5µg/ml to 25µg/ml for vorinostat. The correlation coefficient was found to be 0.999. The Recovery studies were performed for vorinostat in the range of 50% - 150 %. The % Assay for vorinostat is 99.85 % .Forced Degradation studies were conducted according to the ICH guidelines and the Drug Product was found to be stable in all conditions. Hence, the method could be successfully applied for routine analysis of vorinostat capsules.

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INTRODUCTION

Vorinostat also known as suberanilohydroxamic acid (suberoyl+anilide+hydroxamic acid abbreviated as SAHA) is a member of a larger class of compounds that inhibit histone deacetylases (HDAC).N-hydroxy-N'-phenyloctanediamide is the IUPAC name of vorinostat and molar mass is 264.32 g/mol and its molecular formula is $C_{14}H_{20}N_2O_3$.Histone deacetylase inhibitors (HDI) have a broad spectrum of epigenetic activities. Vorinostat is marketed under the name Zolinza (zo-***LINZ***-ah) by Merck for the treatment of cutaneous manifestations in patients with cutaneous T cell lymphoma (CTCL) when the disease persists, gets worse, or comes back during or after two systemic therapies. Vorinostat has been shown to bind to the active site of histone deacetylases and act as a chelator for zinc ions also found in the active site of his tone deacetylases. Vorinostat's inhibition of his tone deacetylases results in the accumulation of acetylated his tones and acetylated proteins, including transcription factors crucial for the expression of genes needed to induce cell differentiation Literature survey reveals chromatographic method [ZOU Qiao-gen, 2011] and [Patel, 2008] lc-ms method for the analysis of vorinostat in pharmaceutical dosage forms and one bioanalytical method for the analysis of vorinostat in rat plasma [Ramesh Mullangi, ?; Elham**,** 2010].

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We here report a totally new, rapid, simple, accurate, precise and linear stability indicating RPHPLC isocratic assay method for the determination of vorinostat in capsules and validate the developed method as per ICH guidelines.

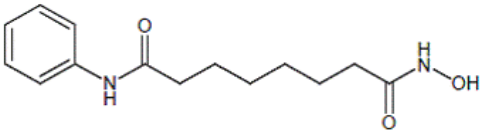

Fig. 1. Structure of vorinostat

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure sample of vorinostat with purities greater than 99% was obtained as gift. Sample from sura labs, Hyderabad, India and capsule formulation [ZOLINZA] was procured from MEDPLUS Pharmacy, Hyderabad, India with labelled amount 100mg of VORINOSTAT. Methanol (HPLC grade), water (HPLC grade), were obtained from SD Fine chemicals (Hyderabad, India), 0.45µm and 0.22 µm Nylon membrane filters wereobtained from Spincotech Private Limited, Hyderabad, India.

Instrument

HPLC analysis was performed on WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector

and a reverse phase C18 column, Apollo C18 (4.6 x 150mm, 5µm).An electronic analytical weighing balance (0.1mg sensitivity, Sartorius, digital pH meter (DELUX model 101), asonicator (sonica, model 2200 MH) and UVVisible Spectrophotometer (Shimadzu UV-1800 series, software-UV probe version 2.42) were used
in this study.

METHODS

Selection of Wavelength

Forced degradation samples, standard and blanks along with controls were injected into HPLC at various wavelengths *viz*. 220nm, 254nm, 280nm and 315nm. Significant impurities and majority of impurities along with the drug were detected at 247nm and hence was chosen as suitablewavelength.

Chromatographic Conditions

The optimized method employs a reverse phase Column, Apollo C18 (4.6 x 150mm, 5µm) C18(150X4.6mm;5µ), a mobile phase of 100% methanol , flow rate of 1 ml/min and a detection wavelength of 247 nmusing a UV detector.

Mobile Phase Preparation

HPLC Grade Methanol select as the mobile phase.

Diluent

Mobile phase was used as a diluent.

Preparation of Stock and Working Standard Solution

Accurately weigh and transfer 10 mg of Vorinostatworking standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of the above Vorinostatstock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol to get 15µg/ml concentration.

Preparation of Stock and Working Sample Solution

Take average weight of one capsule and crush in a mortar by using pestle and weight 10 mg equivalent weight ofVorinostat sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.15ml of Vorinostatabove stock solution into a 10ml volumetric flask and dilute up to the mark with diluent to get target concentration of 15µg/ml of concentration.

RESULTS AND DISCUSSION

Method Development

RP-HPLC isocratic stability indicating assay method was developed keeping in mind the system suitability parameters i.e. Asymmetric factor (A),number of theoretical plates (N), runtime, separation of drug peak from the forced degradants, detection of drug peak along with significant impurities and majority of impurities.

In order to test the applicability of the developed method to acommercial formulation, ZOLINZA was chromatographed at working concentration (15µg/ml) and it is shown in Figure 4. The sample peak was identified by comparing the retention time with the standard drug. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and drug concentration was determined by using the peak area concentration relationship obtained in the standardization step. The protocol affords reproducible assay of the drug in the sample ranging between 90 and 110%, which is the standard level in any pharmaceutical quality control.

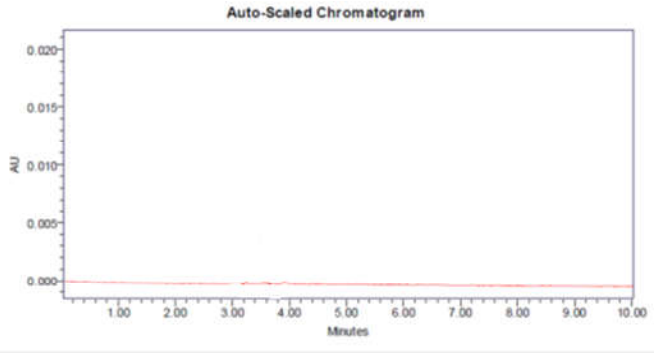

Fig.2: Typical chromatogram of the blank.

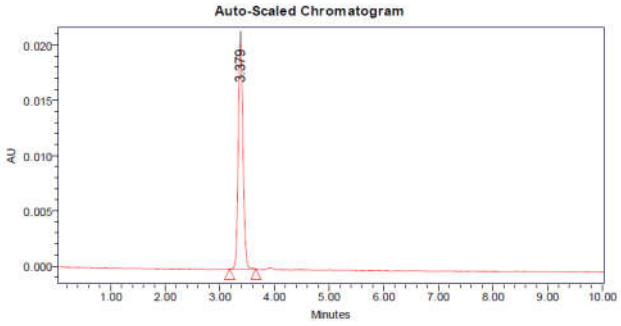

Fig. 3. Typical chromatogram of the standard

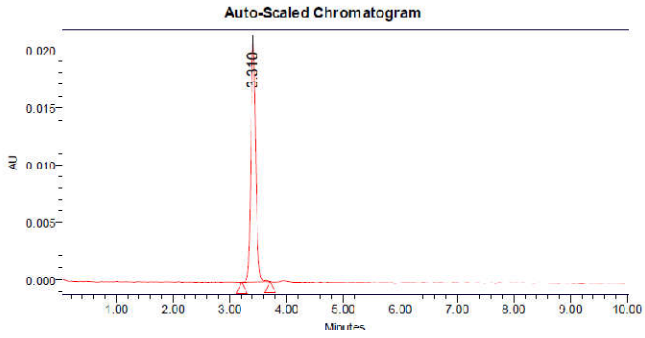

Fig. 4. Typical chromatogram of the control formulation

Method Validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines [5] for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, and sensitivity.

Specificity

Blank, standard drug solution and sample chromatogram revealed that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drug as blank had no peak at the retention time of VORINOSTAT. Accordingly it can be concluded that, the method developed is said to be specific.

Precision

System Precision

Six replicate injections of the standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 1.

Method Precision

Method precision was determined by performing assay of sample under the tests of repeatability at working concentration.

Repeatability (Intraday precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 2).

Intermediate Precision (Ruggedness / Inter day precision)

Six consecutive injections of the sample solution from the same homogeneous mixture at working concentration on a different day by a different analyst, showed % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged (Table 3).

Linearity

Standard solutions of vorinostatat different concentrations level (50%, 75%, 100%, 125% and 150%) were prepared. Calibration curve was constructed by plotting the concentration level of drug versus corresponding peak area.

Table 1. System Precision Results

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vorinostat	3.379	123249	20635	7434	1.10
2	Vorinostat	3.303	123324	20587	7483	1.09
3	Vorinostat	3.322	124060	20690	7550	1.10
4	Vorinostat	3.327	124322	20883	7636	1.10
5	Vorinostat	3.310	123689	20774	7575	1.10
	Average		123728.8		%RSD is less than 2 % .	
	STDEV		462.93487			
	%RSD		0.3741528			

Table 2. Intraday Precision Results

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	% ASSAY
1	Vorinostat	3.397	122374	20741	7605	1.09	98.29715
2	Vorinostat	3.390	122148	20792	7524	1.10	98.11562
3	Vorinostat	3.384	122845	20969	7592	1.11	98.03043
4	Vorinostat	3.378	121881	20889	7585	1.11	97.05618
5	Vorinostat	3.364	121166	20879	7620	1.09	96.9806
	Average		122082.8		%RSD is less than 2%		97.696
	STDEV		622.744490				0.626591
	%RSD		0.51010010				0.641368

Table 3. Intermediate Precision

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	% ASSAY
1	Vorinostat	3.371	123187	20953	7547	1.09	98.9502
2	Vorinostat	3.376	122048	20860	7717	1.09	98.03529
3	Vorinostat	3.382	123165	20800	7557	1.09	98.28579
4	Vorinostat	3.359	121599	20877	7584	1.10	96.83162
5	Vorinostat	3.333	120553	20557	7545	1.09	96.48996
6	Vorinostat	3.341	121567	20798	7496	1.09	98.9502
Average			122110.4	%RSD is less than 2%			97.71857
STDEV			1113.7961				1.028969
%RSD			0.9121222				1.052993

The results show an excellent linear correlation between peak area and concentration level of drug within the concentration range (5-25µg/ml) for the drug and the results are given in Table 4 and Figure 5. The correlation coefficient of vorinostatis 0.999124 and hence the method is said to be linear in the range of 5-25 µg/ml.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 5. The accepted limits of recovery are 90%-110% for the process of determining recovery of the standard from the formulation at three different levels of 50%, 100% and 150%. All observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 4. Calibration Data of vorinostat

N	% Level	concentration(µg/ml)	peak area
1	33.3	5	40088
2	66.6	10	76513
3	100	15	112612
4	133.2	20	143067
5	166.5	25	178937
regression coefficient			0.999124
Intercept			6885.04
Slope			6968.7

Table 5: Recovery Studies Results

% level	sample area	% recovery	Average % Recovery	%RSD
50-1	58422	100.5	100.173	0.306
50-2	57473	99.89		
50-3	55993	100.13		
100-1	112559	99.9	99.4	1.050
100-2	110695	98.2		
100-3	112814	100.1		
150-1	158260	98.17	98.39	0.54
150-2	161104	98.01		
150-3	163872	99.01		

Table 6. Results for degradation studies of Vorinostat

S. no	Type of degradation	Weight of sample (ppm)	Area of sample	Assay content (% w/w)	% Degradation
	Without degradation	15	123249	99	-
1	Acid (0.1N HCl)	15	108756	98.937	0.06
2	Base (0.1N NaOH)	15	105241	95.6242	0.146
3	Peroxide (3% H_20_2)	15	106524	96.833	0.135
4	Thermal (at 70^0 c)	15	109215	99.3	0.113
5	Photolytic (sunlight)	15	107569	97.81	0.127

Sensitivity

The sensitivity of measurement of VORINOSTAT by use of the proposed method was estimated in terms of the limit of quantitation (LOQ) and the limit of detection (LOD). LOQ and LOD were calculated by the use of the equations LOD = 3.3σ/S and LOQ = 10σ/S where σ is the standard deviation of response of calibration plot and S is the slope of the corresponding calibration plot. The limit of detection (LOD) and limit of quantification (LOQ)was found to be 26.100ng/ml and 79.0915ng/mlrespectively.

Forced degradation studies

Sample preparations to perform forced degradation studies

Acid degradation: Take ten average weight of the capsule powder and crush in a mortar by using pestle and weight 10 mg equivalent weight ofVorinostatsample into a 10mL clean dry volumetric flask and add about 3mL of 0.1N HCl and kept a side for 3hours and add 3mL of 0.1N NaOH solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 0.15ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and degassed in a digital ultrasonicator for 10 minutes.

Alkaline degradation

Take ten average weight of the capsule powder and crush in a mortar by using pestle and weight 10 mg equivalent weight ofVorinostatsample into a 10mL clean dry volumetric flask and add about 3mL of 0.1N NaOH and kept a side for 3hours and add 3mL of 0.1N Hcl solution to neutralize the solution and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 0.15ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and degassed in a digital ultrasonicater for 10 minutes.

Peroxide degradation

Take ten average weight of the capsule powder and crush in a mortar by using pestle and weight 10 mg equivalent weight ofVorinostatsample into a 10mL clean dry volumetric flask and add about 3mL of Hydrogen peroxide solution and kept a side for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 0.15ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and degassed in a digital ultrasonicater for 10 minutes.

Thermal degradation

Take ten average weight of the capsule powder and crush in a mortar by using pestle and weight 10 mg equivalent weight of

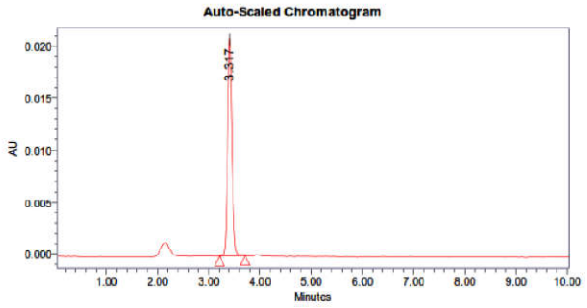

Figure 6. Showing acid degradation

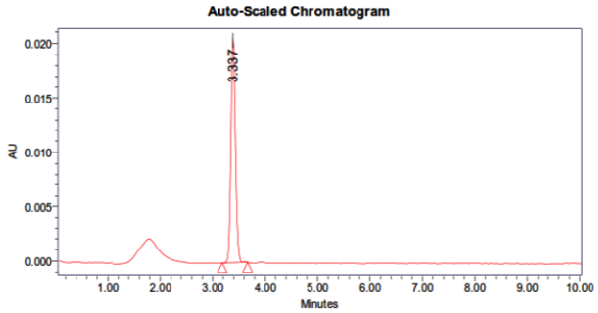

Figure 7. Showing Alkaline degradation

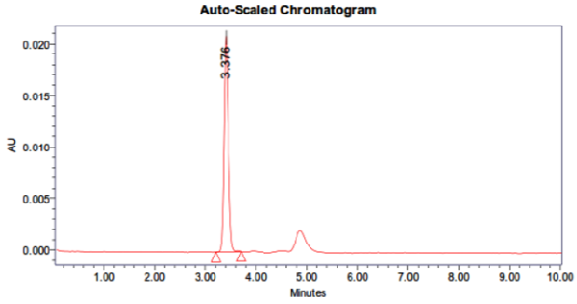

Figure 8: Showing Thermal degradation

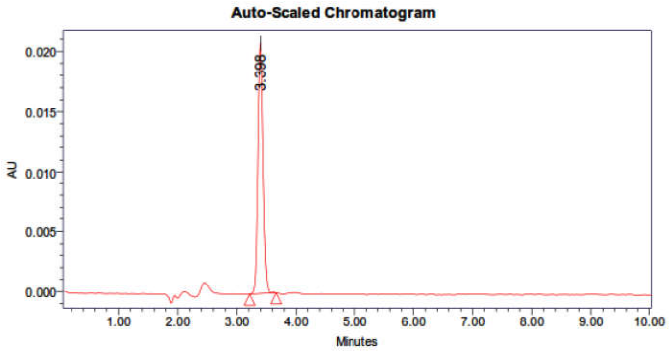

Figure 9. Showing peroxide degradation

Vorinostatsample into a 10mL clean dry volumetric flask and expose to heat at 70ºc for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely. Further pipette 0.15ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and degassed in a digital ultrasonicater for 10 minutes.

Photolytic degradation

Take ten average weight of the capsule powder crush in a mortar by using pestle and weight 10 mg equivalent weight of Vorinostatsample into a 10mL clean dry volumetric flask and expose to sunlight for 3hours and make the volume up to mark by using Diluent and sonicate to dissolve it completely.

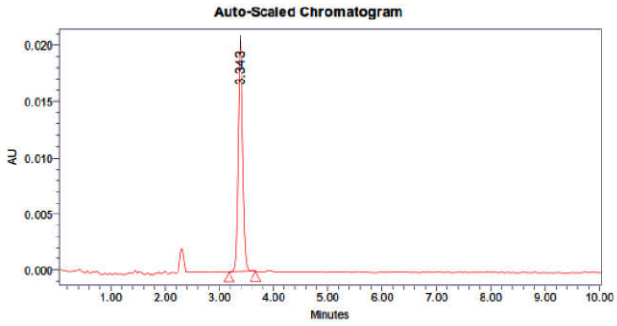

Figure 10: showing Photolytic degradation

Further pipette 0.15ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent and degassed in a digital ultrasonicater for 10 minutes.

Acid degradation

Degradation was observed by the additon of 0.1 N HCl

Conclusion

A reverse phase HPLC isocratic stability indicating assay method has been developed and validated as per ICH guidelines for the quantitative estimation of VORNOSTAT in capsules Forced degradants were separated from the drug peak using the optimized method. Intra day and Inter day precision were exemplified by relative standard deviation of 0.6 and 1.5% respectively. A good linear relationship was observed for the drug between concentration ranges of 5 and 25µg/ml. Accuracy studies revealed that mean recoveries were between 90 and 110%, an indicative of accurate method. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 26.100ng/ml and 79.0915ngrespectively. Accordingly it can be concluded that the developed reverse phase isocratic HPLC stability indicating assay method is sensitive, accurate, precise and linear and therefore the method can be used for the routine analysis of vorinostat in capsules.

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